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Burrowing and soil consumption by earthworms in limed and unlimed soils from *Picea sitchensis* plantations

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With 4 Figures

1. Introduction

In the UK, deep peats in the uplands are often afforested with *Picea sitchensis* ((Bong.) Carr. [Sitka spruce]. These soils typically have a low nitrogen (N) mineralisation rate (McIntosh, 1983), since large amounts of N are locked up in recalcitrant organic matter (Heal *et al.*, 1982). This results in severely nitrogen-limited tree growth (Taylor & Tabbush, 1990). Earthworms, which are known to promote N mineralisation in forest soils (Anderson *et al.*, 1983, Scheu, 1987; Haimi & Huhta, 1990; Robinson, 1990), are scarce in the acidic soils in which *P. sitchensis* normally grows, but abundant in base-rich soils (review by Satchell, 1983). By liming nutrient-poor afforestation sites, earthworm populations could be favoured due to increased reproduction and immigration, together with lower mortality (review by Persson, 1988). Earthworm inoculation into limed forest soils, as performed by Huhta (1979), could accelerate colonisation by earthworms, and allow the establishment of species which are not normally found in coniferous plantations. A greater earthworm biomass in these soils may increase N mineralisation and consequently tree growth.

Liming may become an important way of ameliorating the acidification of forest soils from natural and anthropogenic sources, yet the addition of lime has often yielded disappointing results, with negative tree growth effects, sometime extending over decades (Dickson, 1984). The reduction in tree growth may be due to immobilisation of N in soil organic matter (Popović *et al.*, 1988). This immobilisation could be reduced in the presence of earthworms, and tree production perhaps increased.

The effects of liming coniferous soils on burrowing and soil consumption by earthworms are largely unknown, and must be assessed in preliminary experiments before field inoculation can be carried out. This paper reports on two experiments to determine whether earthworms will enter and consume limed soils taken from stands of *P. sitchensis*. The aim of the first experiment was to determine whether earthworms would burrow into limed (artificially high *p*H) and unlimed (naturally low *p*H) soils from a *P. sitchensis* plantation, and a loam reference soil (naturally high *p*H) when (a) restricted to one soil type, and (b) allowed a choice between them. The aim of the second experiment was to determine the extent to which the different earthworm species would consume limed peat. On the assumption that the great majority of ingested material passes unabsorbed through the gut, faecal production on starvation can be approximately equated with the soil content of the alimentary canal (Piearce, 1972a).

2. Materials and methods

2.1. Soil discrimination tests

The soil used was obtained from a small, well-established (approximately 40 years old) *Picea sitchensis* plantation on the University of Lancaster campus, where there is a deep (ca. 10 cm) organic horizon, overlying an eluviated horizon (AVERY, 1980). The *p*H values of the test soils in water were 3.9 and 3.8 for the F and Ea horizons, respectively. A subsample of each horizon was mixed with agricultural ground limestone (1 g limestone with approximately 50 g F, and 5 g Ea, horizon soils, respectively) equivalent to a field rate of 10 t ha^{-1}. The lime was mixed in thoroughly with the soils, which were left overnight before use to facilitate neutralisation. The reference soil, a loam (*p*H 7) with a good crumb structure, was collected from under grass. All soils were crumbled to ensure similar physical properties between them, and were moistened with distilled water before use.

Choice chambers were set up by dividing appropriate containers in half with removable plastic barriers. Petri dishes, 9 cm in diameter, were used in the trials with *Allolobophora chlorotica* (SAVIGNY) and *Dendrodrilus rubidus* (SAVIGNY) and plastic dishes, 20 cm in diameter and 6 cm in depth, were used for trials with *Aporrectodea caliginosa* (SAVIGNY), *Ap. longa* (UDE), *Lumbricus terrestris* L. and *L. rubellus* HOFFMEISTER. One side of the container was filled with a test soil, and the other with the reference soil. The volume of soil used to fill half of the container was approximately 30 cm^3 for the Petri dish or 1 dm^3 forthe larger plastic dish. Limed or unlimed F or Ea soils were tested against the reference soil with four replicases of each combination.

Aporrectodea caliginosa and *A. chlorotica* were collected by hand-sorting soil from a stand of *Populus alba* L., and a mixed deciduous woodland, respectively. *Aporrectodea longa* and *D. rubidus* were hand-sorted from a compost heap and well decayed horse manure, respectively. *L. rubellus* was hand-sorted from a mixed deciduous woodland soil and well decayed sewage sludge, and *L. terrestris* was expelled from fertile grassland using a 0.25% formaldehyde solution. Individuals of *L. terrestris* were washed immediately after emergence and allowed to recover in grassland soil for five days prior to use in experiments. The other species were stored in the soils from which they had been collected, to which compost was added as an additional source of food, prior to being used experimentally.

The same number of worms was placed on the soil on each side of the barrier (4 individuals for *D. rubidus*, 4 individuals for *A chlorotica*, 2 of which were the green morph and 2 of which were pink, and 3 individuals for the other species) and the chamber was left uncovered at approximately 20 °C under standard fluorescent lighting (100 lux) 2 m above the chamber. Limed and unlimed trials for a particular species were carried out at the same time and under the same environmental conditions. The numbers of earthworms on each soil surface were counted after 1, 2, 5, 10, 15, 20, 30 and 40 min. Disappearance was measured to ensure that the earthworms could move freely into the soils under these conditions, and that when given the choice between two soils there were not physical factors (e.g. compaction) greatly prohibiting movement into one soil. After 40 min, the barrier was removed, and the container covered and left in the dark for 4 h. The number of earthworms on each side was then counted. Numbers were summed for the four replicates. Soil moisture contents, determined by drying samples at 105 °C overnight, and *p*H values, measured using a 1:3 (v/v) soil:water slurry, were determined for limed, unlimed and reference soils during the trials with *L. rubellus*. The number of earthworms left on the surface was compared between the test and reference soils for the time period when 50% of the earthworms had burrowed into the reference soil, by applying Fisher's Exact test. The differences in numbers of earthworms in each soil after 4 h were assessed using the χ^2 test and *p*H values were compared using t-tests.

2.2. Consumption of limed peat

The site chosen for the second part of this study is part of Kershope forest, Cumbria (National Grid Reference NY566320), planted in 1969 with *Picea sitchensis*. The Kershope site was also used in a later part of the project to assess the effect of earthworm inoculation and liming on nutrient mineralisation rates (ROBINSON 1990). At the site, situated on non-flushed slope peat bog (PYATT, 1970) over Carboniferous limestone, an aerial application of P at 75 kg ha^{-1} and K at 100 kg ha^{-1} was made in 1983. The native vegetation outside the canopy is *Calluna vulgaris* (L.) HULL, *Eriophorum vaginatum* (L.) and *Sphagnum* spp.. Blocks of fresh peat, without surface accumulations of *P. sitchensis* litter, were collected from approximately 3 m outside the *P. sitchensis* canopy and sealed inside plastic

bags. The peat monoliths were taken from outside, rather than inside, the forest canopy, since if earthworms were to be applied at first rotation planting, there would be no surface accumulation of *P. sitchensis* litter for them to feed on. The peat blocks were stored at 1 – 2 °C before use. In the laboratory, these blocks (*p*H range 3.6 – 4.7) were trimmed to a depth of 5 cm, and the live native vegetation and woody roots removed.

This experiment comprised two treatments of limed and unlimed peat. One gram of ground limestone was mixed with the equivalent of 6.45 g dry mass of peat (about 25 to 60 g fresh mass of peat, depending on the moisture content); this treatment was approximately equivalent to 10 t ha^{-1} lime. The lime was mixed thoroughly with the peat to ensure rapid neutralisation of the soil acidity, and to provide a more appropriate food source for the earthworms than if lime was simply spread on the soil surface. All soils were crumbled, thoroughly mixed and used to fill plastic pots (10 cm × 7 cm, height × top diameter, volume 250 cm^3). Bulked samples of live surface vegetation, 2 g fresh weight, were added to each container for both limed and unlimed treatments, and all containers were then left at 2 °C for 24 h before use.

Earthworms (*Ap. caliginosa*, *L. rubellus*, *L. terrestris* and *D. rubidus*) were collected and stored as described previously. Each individual worm was washed free of adhering soil, and placed on damp tissue paper for 48 h in the dark at 10 °C, before being introduced into the experimental pot. During this procedure gut contents were largely evacuated. One adult and one immature earthworm of a single species were placed in each container. Adults and immatures were used, since they exhibit different feeding patterns; the immatures feed and grow more rapidly (DICKSCHEN & TOPP, 1987). Six replicates were set up for each species and each treatment. The pots were left at 10 °C in the dark for 48 hours, when the earthworms were removed by hand-sorting. Moisture contents and *p*H were measured as before. Each worm was washed, gently dried on tissue paper, weighed and placed separately in a Petri dish, with a drop of water to prevent dehydration. The earthworms were kept at 10 °C in the dark for 48 h. Faecal matter was air-dried, weighed and examined microscopically to determine the nature of the material consumed in the limed and unlimed treatments. Faecal production figures were taken as the dry mass of faeces produced (during 48 h in a Petri dish) per unit fresh body mass of starved earthworm after the earthworms had been kept in peat for 48 h.

Faecal production figures for limed and unlimed peats were compared for each species by applying t-tests after square root transformation. A factorial analysis of variance was carried out on the combined consumption data for the 4 worm species and the 2 rates of liming. Tukey's Honestly Significant Difference Comparison of means was used as an *a posteriori* test to identify significant differences in the species' faecal outputs. Moisture contents and *p*H values were compared using t-tests.

3. Results

3.1. Soil discrimination tests

Soil moisture contents and *p*H values are shown in table 1. Percentage disappearance into limed and unlimed F horizon soils was generally similar to that into the reference loam, however *Ap. caliginosa* ($p < 0.001$, fig. 1(a)) and *Ap. longa* burrowed much more

Table 1. Moisture contents and *p*H values for burrowing/soil selection trials (mean of 4 replicates).

Soil type	Mean moisture content (% of soil dry mass ± S.E.)	Mean *p*H
Reference soil	49.5 ± 1.0	6.66***
Unlimed F horizon	350.5 ± 19.3	3.80
Reference soil	54.9 ± 3.1	6.41***
Limed F horizon	309.8 ± 25.5	7.34
Reference soil	50.5 ± 1.6	6.84***
Unlimed Ea horizon	69.4 ± 2.0	3.57
Reference soil	55.9 ± 1.7	6.41***
Limed Ea horizon	86.2 ± 1.8	7.18

***, $p < 0.001$, *p*H of reference vs test soil, t-test.

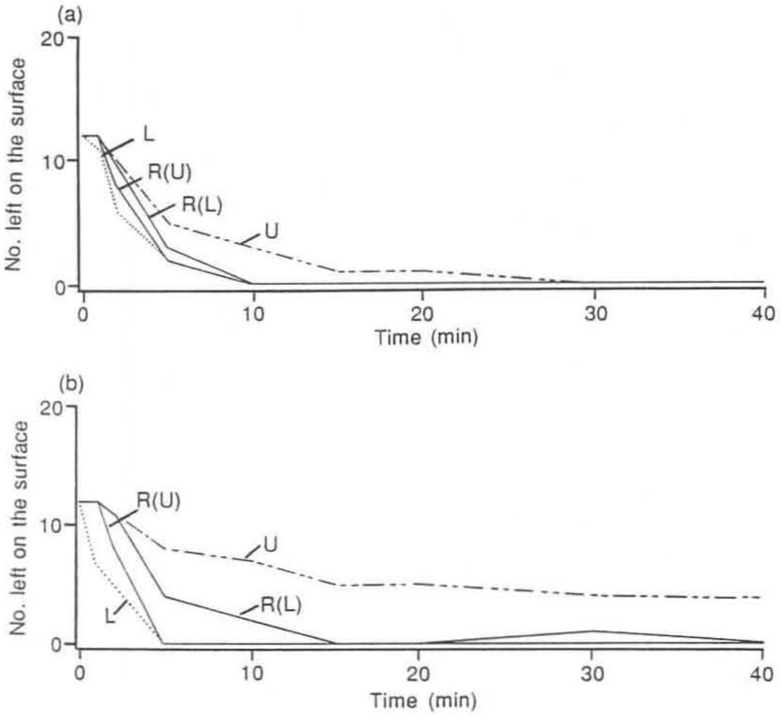

Fig. 1(a) Disappearance of *Ap. caliginosa* into reference (R), limed (L) and unlimed (U) *Picea sitchensis* F horizon soils. R(L) = reference soil for limed trial, R(U) = reference soil for unlimed trial.

Fig. 1(b) Disappearance of *L. rubellus* into reference, limed and unlimed *P. sitchensis* Ea horizon soils. Key as Fig. 1(a).

slowly into the unlimed coniferous soil than the loam and *L. rubellus* disappeared markedly more quickly in limed F horizon soil than than the loam ($p < 0.05$). Earthworms generally burrowed as quickly in the limed Ea horizon soil as in the loam; significantly more rapidly for *L. rubellus* ($p < 0.001$, fig. 1(b)) and *D. rubidus* ($p < 0.05$). All species disappeared less rapidly in unlimed Ea horizon soil than reference loam, although for no species was this trend statistically significant.

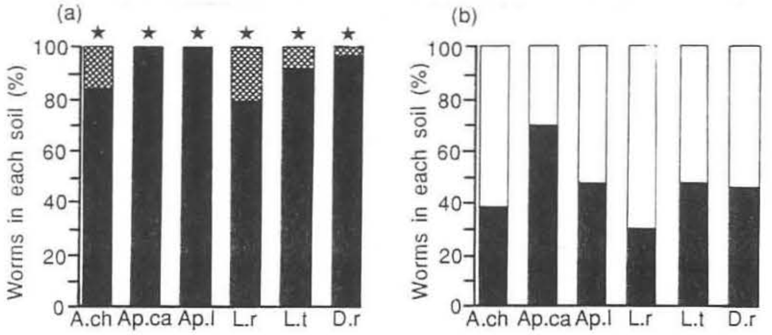

Fig. 2. Soil selection by earthworms. Percentage in each soil after 4 hours. A.ch. = *A. chlorotica*, Ap.ca = *Ap. caliginosa*, Ap.l. = *Ap. longa*, L.r = *L. rubellus*, L.t = *L. terrestris* and D.r. = *D.rubidus*. *, $p < 0.001$, test soil v reference, χ^2 test.
(a) Reference loam (shaded) v unlimed *P. sitchensis* F horizon soil (hatched);
(b) Reference loam (shaded) v limed *P. sitchensis* F horizon soil (unshaded).

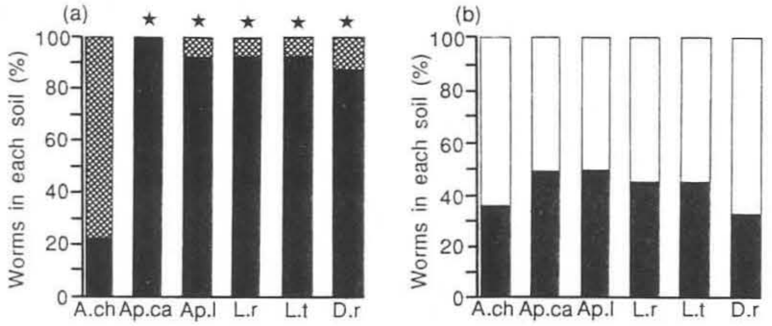

Fig. 3. Soil selection by earthworms. Percentage of earthworms in each soil after 4 hours. Key to symbols as Fig. 2.
(a) Reference loam (shaded) v unlimed *P. sitchensis* Ea horizon soil (hatched);
(b) Reference loam (shaded) v limed *P. sitchensis* Ea horizon soil (unshaded).

The numbers of earthworms in the reference soil compared to the unlimed F horizon soil after 4 hours (fig. 2a) suggest a strong preference in all species for the loam. For all 6 species tested, once the F horizon soil was mixed with lime, there was a more even distribution of earthworms between the two soils (fig. 2b). The pattern was similar for the Ea horizon soil (figs. 3a, b), however, more *A. chlorotica* individuals were found in the unlimed Ea horizon soil rather than the loam (fig. 3a). In this case the worms were often all wrapped together in a ball in the unlimed soil, which is a stress response. With limed Ea soil, there was a more even distribution of worms (fig. 3b).

3.2. Consumption of limed peat

Table 2 shows mean soil moisture contents and *p*H values for the six replicates. All 4 earthworm species consumed more peat in the limed treatment (fig. 4). Consumption of both limed and unlimed peat by the 4 species was significantly different (ANOVA, $p < 0.05$), *L. rubellus* consuming significantly more peat than *D. rubidus* (Tukey's HSD, $p < 0.05$).

Table 2. Moisture contents and *p*H values for each soil type used in the feeding trials (mean of 6 replicates).

Species	Peat type	Mean moisture content (% of soil dry mass ± S.E.)	Mean *p*H
Ap. caliginosa	Limed	503 ± 11	7.11***
	Unlimed	629 ± 93	4.43
L. rubellus	Limed	568 ± 12***	7.11***
	Unlimed	715 ± 16	4.55
L. terrestris	Limed	213 ± 6***	6.53***
	Unlimed	275 ± 8	3.77
D. rubidus	Limed	556 ± 47	7.08***
	Unlimed	690 ± 44	4.38

***, $p < 0.001$, limed vs unlimed, t-test.

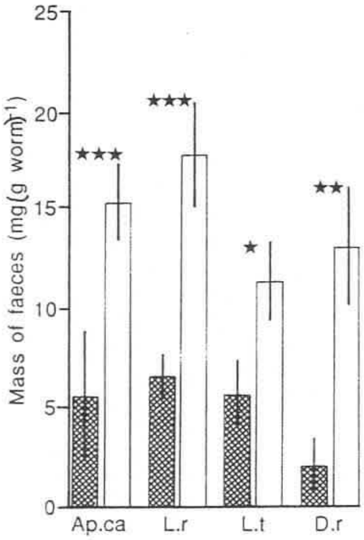

Fig. 4. Faeces produced by earthworms after being kept in limed (unshaded) and unlimed (hatched) peat. Mean air-dried mass as percentage of earthworm fresh mass, ± S.E.. Ap.ca = *Ap. caliginosa*, L.r = *L. rubellus*, L.t = *L. terrestris* and D.r. = *D. rubidus*. *, **, ***, $p < 0.05$, 0.01, 0.001, limed v unlimed, t-test.

4. Discussion

On the basis of the preference tests, liming significantly increased the acceptability of *Picea sitchensis* soils to all 6 species of earthworm tested, whether they were acid intolerant (*A. chlorotica*, *Ap. caliginosa*, *Ap. longa*) or ubiquitous (*L. rubellus*, *L. terrestris*, *D. rubidus*) *sensu* SATCHELL (1955). The most obvious effect of liming was to increase soil *p*H (table 1). However, there are other possible influences of liming to consider, for example modification of the physical nature of the soil by lime which might increase the ease with which earthworms can burrow. The comparatively rapid rate of disappearance of earthworms from the surface of all soils suggests that differences in numbers in each soil type when given the choice are unlikely to be due to differences in penetrability.

The differences in moisture content between the reference soil and the *P. sitchensis* soils used in the preference tests (table 1), are unlikely to have had any appreciable effect on overall earthworm distribution. The moisture contents of the soils used were well within the tolerance ranges of all the earthworm species, and there was no discernible relationship between soil moisture and earthworm numbers in these trials.

Liming signifcantly increased the peat content of the gut in all 4 species (fig. 4). No allowance was made for the weight of lime ingested (which could have contributed to a greater density and hence greater weight of faeces produced from the limed treatment). However, from microscopic examination of the faeces it was clear that consumption of organic matter had been substantially increased in the limed compared to the unlimed treatment, and a correction was not deemed necessary. In using the quantity of material egested as a measure of that ingested by earthworms, it has been assumed that losses due to assimilation are not appreciably different for diets of limed and unlimed peat. Assimilation effeciencies recorded by earthworms are generally low (LEE 1985; but see DICKSCHEN & TOPP, 1987), as for other detritus feeders e.g. diplopods (MCBRAYER, 1973) and isopods (RUSHTON & HASSALL, 1983).

The greater amount of soil consumed in the limed treatment is consistent with the observation of SATCHELL & DOTTIE (1984), that in acid peats (*p*H 3.6–4.3) *Ap. caliginosa*

produced less faeces and burrowed less than in more neutral peats (pH 5.5 – 7.2). In their study, faecal weight was significantly positively correlated with pH for *Eisenia fetida* SAVIGNY living in an organically rich medium. Low pH has been shown to stimulate nervous activity in the body wall of the earthworm (LAVERACK, 1961). On markedly acid media earthworms frequently show signs of surface irritation, for example, exudation of mucus and coelomic fluid, together with rapid movement. The irritating effect on the integument and the nature of the acid medium as food may limit feeding activity.

Over a short time-scale, all species of earthworm tested moved preferentially into limed soil from *P. sitchensis* plantation, and consumed more limed than unlimed peat. On the basis of the results, none of the earthworm species can be ruled out as candidates for field inoculation, and indeed, it may be possible to use species which are rare in typical coniferous soils e.g. *Ap. caliginosa* and *Ap. longa* (PIEARCE, 1972b; NORDSTRÖM & RUNDGREN, 1974). It would appear that limed coniferous soils are more appropriate for inoculation of earthworms than unlimed ones, although it is necessary to test whether the earthworms can survive and grow in the long-term in limed coniferous soil before inoculation is carried out (ROBINSON *et al.*, in press).

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Earthworms of 6 species burrowed rapidly into limed *Picea sitchensis* F and Ea horizon soil, and less rapidly into unlimed soils. In selection tests unlimed *P. sitchensis* soils were avoided, but there was generally no discrimination against limed soils. All 4 species tested consumed more limed than unlimed peat. The species investigated can therefore be considered as possible candidates for inoculation into limed deep peat soils under *P. sitchensis* to improve rates of N mineralisation, and enhance tree growth.

Keywords: Earthworms, lime, *Picea sitchensis*, N mineralisation, *p*H, preference test, peat consumption.

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